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PHYTOCHEMICAL SCREENING AND PROTECTIVE EFFECT OF ADHATODA VASICA LEAF EXTRACTS AGAINST FREE RADICAL DAMAGE

Nagesh Ramya [1], Nagarathna Amresh [2], Kiruthika Balasubramanian [3]

[1]Department of Community Medicine, Sri Devaraj Urs Medical College (SDUMC),Sri Devaraj Urs Academy of Higher Education and Research (SDUAHER), Tamaka, Kolar, Karnataka, India
[2]Principal, M.S. Ramaiah College of Arts, Science and Commerce, Bengaluru, Karnataka, India
[3]Assistant Professor ,Department of Biochemistry M.S. Ramaiah College of Arts, Science and Commerce, Bengaluru, Karnataka, India

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Abstract:

Background: *In the past few decades there has been assumption on the medicinal values and the beneficial potential of medicinal plants in therapeutic components. Cellular metabolism produces Reactive Oxygen Species (ROS), free radicals' forms cascade leading to homeostatic disruption of living tissues. The shift in balance between oxidants and antioxidants in favor of oxidants is termed as 'oxidative stress', leading to chronic and degenerative disorders.*

Objective: *The present study was undertaken to analyze the antioxidant's free radical scavenging effects of Adhatoda vasica leaves and to screen the phytochemicals of the plant.*

Methodology: *The three solvents extract with increasing polarity (Hexane, Ethylacetate and Isopropanol) were prepared by following successive extraction. The scavenging effect of extracts against free radicals like hydrogen peroxide radical and hydroxyl radicals was analysed. The chelating property and phytochemicals of the plant extract was screened. The DNA protective action against oxidative damage was also analysed.*

Results: *Isopropanol and ethyl acetate extracts of the leaves exhibited high levels of hydrogen peroxide and hydroxyl radical scavenging activity followed by hexane. All the three extracts showed significant chelating property. The Adhatoda vasica exhibited protective action against oxidative damage caused by the free radicals. The leaves indicated the presences of alkaloids, phenolics and flavonoids.*

Conclusion: *Normally body can fight radicals by means of antioxidant system. The radical scavenging effects of plant plays major role in protecting living organisms against damage. Adhatoda vasica leaf extract revealed the effective protection of DNA against radicals.*

Keywords: *Adhatoda vasica, Radical Scavenging, Phytochemicals, Chelating property, Antioxidants.*

Corresponding author:

Kiruthika Balasubramanian,
Assistant Professor, Department of Biochemistry,
M.S. Ramaiah College of Arts, Science and Commerce,
Bengaluru, Karnataka,
India, Pin code - 560054,
Email: krithikagandhii@gmail.com

QR code

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INTRODUCTION:

In India many Ayurveda practitioners are using various indigenous plants for the treatment of different disease and conditions.[1] Medicinal plants are used as raw materials in extraction of active components that can be used to produce and develop different therapeutic drugs.[2] In the past few decades there has been assumption on the medicinal values and the beneficial potential of medicinal plants in therapeutic components.[3]

The cellular metabolism produces Reactive Oxygen Species (ROS) and the mitochondrial cells uses oxygen to generate energy giving rise to unstable free radicals. Depending on the concentration and environment the radicals can be beneficial and harmful to the tissues.[4,5] The free radicals like hydrogen peroxide, hydroxyl radical, superoxide ion, oxygen singlet etc., attacks the other molecules in cells and forms a cascade of reaction (chain) leading to cell damage and homeostatic disruption of living tissues.[6,7] The antioxidants combat free radicals and terminate the chain before vital molecules are damaged.[8]

The shift in balance between oxidants and antioxidants in favor of oxidants is termed as 'oxidative stress', which leads to chronic and degenerative disorders. The endogenous and exogenous antioxidant enhances the immune defense and lowers the risk of diseases by scavenging the free radicals and blocks the harmful effects of ROS.[4,9]

Phytochemicals are bioactive molecules called as secondary metabolites which contributes to disease prevention and promotes health; they have recently emerged as modulators of key cellular signaling pathways. Phytochemicals suppress the cell proliferation and blocks the mutagenic activity of carcinogens, protects against lipid peroxidation and inflammatory responses.[10–12] In the modern world multiple drug resistance has developed against microbial infections due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease.[13] In pharmaceutical field medicinal plants are mostly used for the wide range of substances present in plants which have been used to treat chronic as well as infectious diseases.[14]
The herb *Adhatoda vasica* is used in herbal medicines. The plant as a whole are used to relieve cough, cold, whooping-cough, asthma and bronchitis due to their pharmacological properties. The extract from leaves has been used to relieve asthma, bronchitis, breathlessness, urinary infection for centuries. It is also used to relieve other conditions like local bleeding due to piles, peptic ulcers and menorrhagia; and relief from bleeding gums and pyorrhea. Crushed leaves are applied to relieve conditions like skin ailments, worms and rheumatoid arthritis. Warm crushes are effective to relive dislocated joint and rheumatic pain. It is also an effective expectorant to relieve congestion and dyspnea.[15]

The present study was undertaken to analyze the antioxidant, free radical scavenging effects of the leaves of *Adhatoda* and to screen the phytochemicals of the plant.

2. METHODOLOGY:

2.1 Preparation of plant extract

In our study the leaves of *Adhatoda vasica* was used. The plant with its fresh leaves were collected from University of Agricultural Science, Gandhi Krishi Vignana Kendra (GKVK), Hebbal, Bangalore. The fresh leaves were washed under running tap water to free from the surface contaminants (like mud and dust) and pat dried the leaves on layers of soft tissue papers.

The leaves of *Adhatoda vasica* were air dried in shade at room temperature without exposing them to sunlight for 10 days. The completely dried leaves were then grinded to fine powder and weighed. The different solvent extracts were prepared by following Successive Extraction procedure

2.1.1 Successive extraction

10g of powdered leaves was dissolved in 100ml of hexane and placed in shaker incubator for 24 hours. The solvent was filtered and the filtrate was stored. This filtrate forms the hexane extract. The residue was air dried, weighed and again dissolved in 100ml of Ethyl acetate solvent, kept for incubation for 24 hours in shaker incubator. The solvent was then filtered and stored, which serves as Ethyl acetate extract. The leftover residue was air dried and weighed which was finally dissolved in Isopropanol solvent and carried out the same procedure. All the three solvent extracts (Hexane, Ethyl acetate and Isopropanol) were then dried (allowed the solvent to evaporate) and the leftover dried residues were the forms of sample used for the assays.

These extracts were used to determine free radical scavenging effect, Chelating properties, phytochemical screening and the protective action against free radical damaging the goat liver DNA.

2.2 Radical scavenging effects of *Adhatoda vasica*

The free radical scavenging effects of the leaves were analysed against hydrogen peroxide (H_2O_2) and Hydroxyl radicals. The H_2O_2 scavenging ability of the extracts of the leaves was determined according to the method of Ruch et al.[16] The scavenging capacity for hydroxyl radical was measured according to the method of Elizabeth et

al.[17] The chelating property of *Adhatoda vasica* leaf extracts was determined based on the spectral changes following the method of Brown et al.[18]

2.3 DNA protective effect of *Adhatoda vasica*

Goat liver DNA was isolated by the method of Marmur. [19] 1% agarose gel was prepared and the samples were loaded by mixing 2µl of 6x gel loading dye to 15 µl of DNA as control DNA. 15 µl of different plant extracts were mixed with 15 µl of DNA along with 6x gel loading dye and loaded in respective wells. DNA was induced to damage by generating free radical by addition of hydrogen peroxide and loaded in respective wells. DNA with extracts and hydrogen peroxide were then mixed and loaded into well for the migration pattern. The gel electrophoresis was carried out in 100volts.

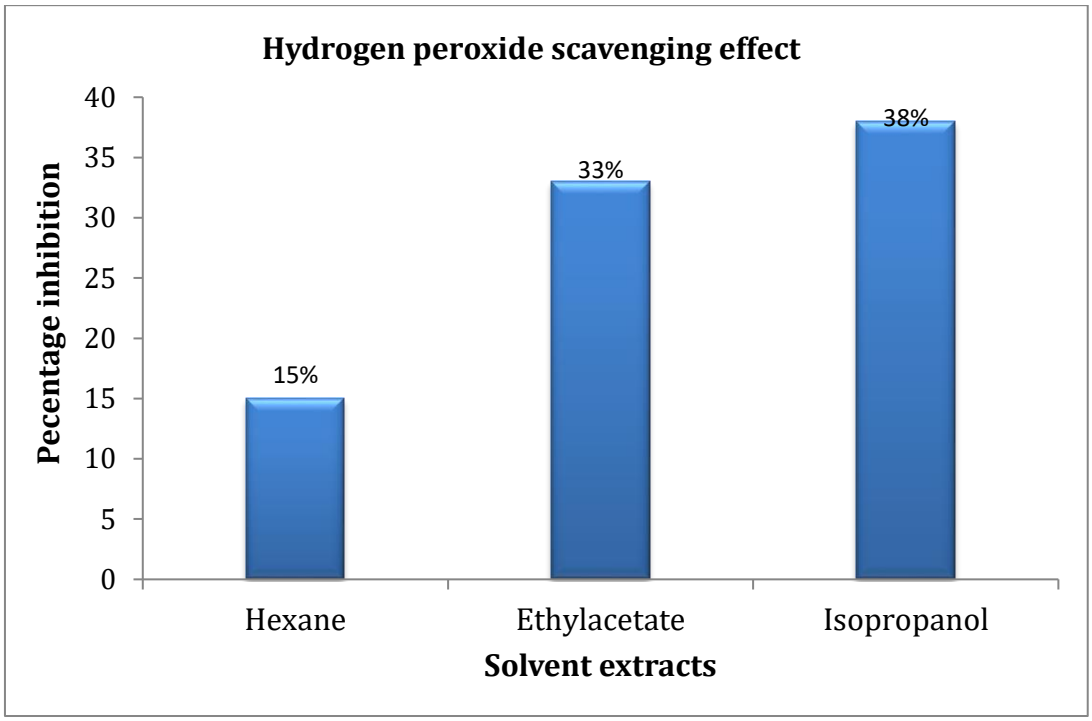
2.4 Preliminary phytochemical screening of *Adhatoda vasica* leaves

The extracts of *Adhatoda vasica* leaves were tested for the presence of various phytochemicals as described by Khandelwal.[20] Alkaloids Mayer's test, Dragendroff's test and Wagner's test were used to detect Alkaloids. Phenolics was analysed using ferric chloride test and lead acetate test. To detect flavonoids aqueous sodium hydroxide test, sulphuric acid test were used.

3. RESULTS

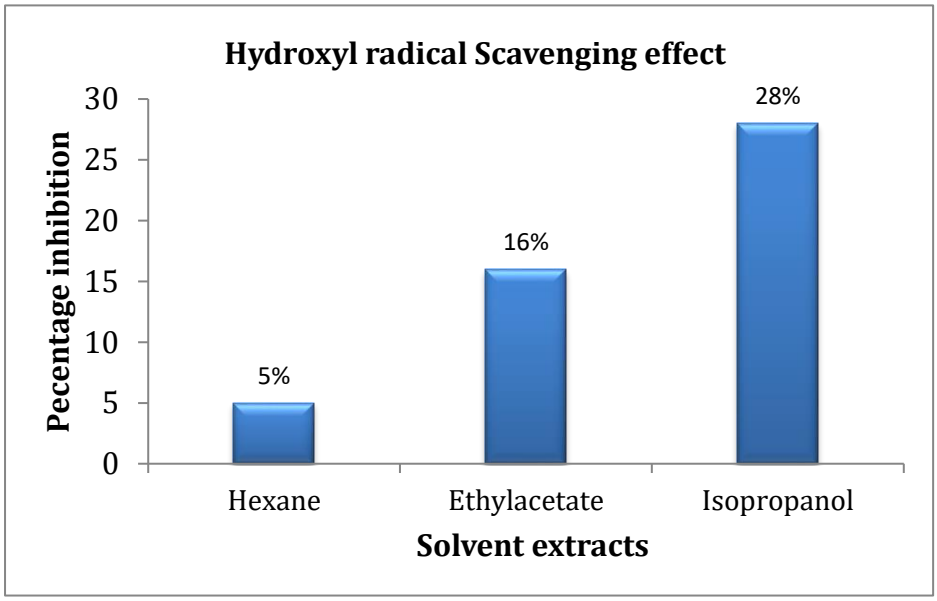
The leaves of medicinal plant *Adhatoda vasica* showed the following results of radical scavenging activity. The results showed that the isopropanol and ethyl acetate extracts of the leaves exhibited high levels of hydrogen peroxide scavenging capacity followed by the hexane extract. The result of hydrogen peroxide scavenging is depicted in Figure 1. The isopropanol extracts exhibited 38% followed by ethylacetate 33%. The hexane extract showed only 15%, which indicate that the phytochemicals responsible for the antioxidant property are not non-polar in nature.

Figure 1: The graph depicts the hydrogen peroxide radical scavenging effect of *Adhatoda vasica* leaves in three different solvent extracts Hexane, Ethylacetate and Isopropanol (increasing polarity)



The results of hydroxyl radical scavenging ability of the *Adhatoda vasica* leaves showed the same trend as that of hydrogen peroxide scavenging activity. The results are presented in Figure 2. The isopropanol, ethylacetate and hexane extract showed 28, 16 and 5 % hydroxyl radical scavenging ability respectively.

Figure 2: The graph presents the hydroxyl radical scavenging effect of *Adhatoda vasica* leaves in three different solvent extracts with increasing polarity Hexane, Ethylacetate and Isopropanol.



The chelating property of three different extracts is represented in Figure 3. The ethyl acetate and isopropanol extracts showed almost equal percentage of chelating property (50 and 49 %) followed by hexane extract (36%).

Figure 3: The graph shows the chelating property of *Adathoda vasica* leaves in three different solvent extracts Hexane, Ethylacetate and Isopropanol having increasing polarity

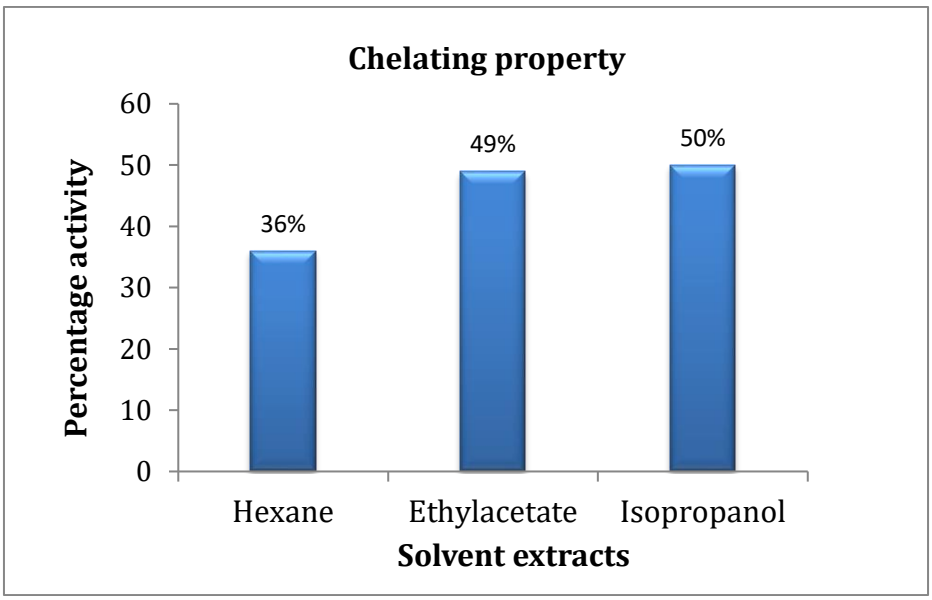
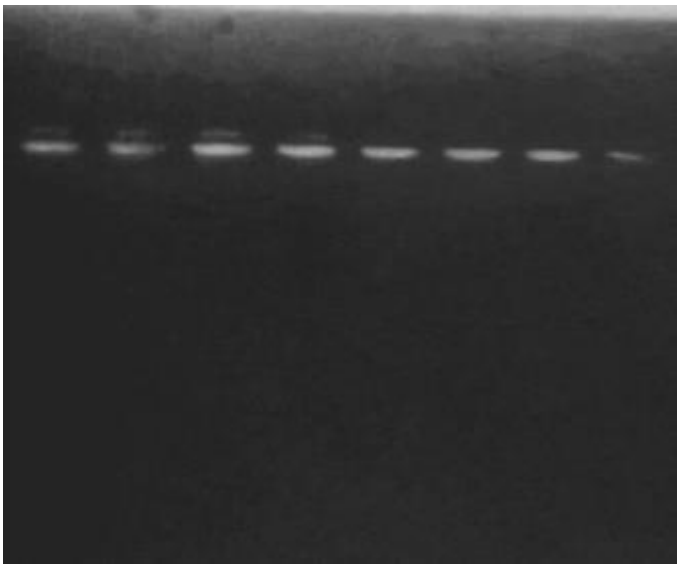


Figure 4: Picture of agarose gel electrophoresis of goat liver DNA. The *Adhatoda vasica* leaf extract exhibited positive results in protecting the DNA against free radical generated by hydrogen peroxide.



Lane1- DNA + Hexane extract + H_2O_2; Lane2- DNA + Ethyl acetate extract+ H_2O_2; Lane3- DNA + Isopropanol extract+ H_2O_2; Lane4-DNA + Hexane extract; Lane5- DNA + Ethyl acetate extract; Lane6- DNA + Isopropanol extract; Lane7- DNA; Lane8- DNA + H_2O_2

The effect of *Adhatoda vasica* leaves on Oxidative damage is presented in figure 4, which shows the migration pattern of hydrogen peroxide treated DNA in the presence and absence of leaf extracts. As can be seen from the picture, H_2O_2 caused extensive damage to goat liver DNA (lane 8). The leaf extracts, by themselves, did not cause any damage to DNA (lanes 4, 5 and 6). Additionally, the presence of the extracts caused a significant protection to DNA, signified by the presence of intact bands (lanes 1, 2 and 3). The *Adhatoda vasica* leaf extract exhibited positive result in protecting the goat liver DNA against radical generated by hydrogen peroxide. The results of the preliminary analysis of the phytochemicals of the *Adathoda vasica* leaves indicated the presences of alkaloids, phenolics and flavonoids.

4. DISCUSSION

In our study the leaves of *Adathoda vasica* was selected to evaluate its free radical scavenging effects and antioxidant capacities at three different polarities. The analysis resulted in showing that the plant is a good source of antioxidants and has the ability to protect the cells and tissues from free radicals. There are various other studies on plants which support our study results.

Hydrogen peroxide gives rise to hydroxyl radical and this serves two contradicting functions, one which damages the cells by lipid peroxidation, by inactivating the enzymes and another serves as an inducer for antioxidants enzymes.[21] The water and ethanol extracts of *Crataegus monogyna* were capable and exhibited the scavenging activity on hydrogen peroxide.[22] Our results shows that the isopropanol and ethyl acetate extracts of the leaves exhibited high levels of hydrogen peroxide scavenging capacity.

Hydroxyl radical is biologically more reactive and causes hydroxylation of biomolecules by reducing the unsaturated bonds in their structure and most of the natural substances are endowed with effective radical scavengers.[23] In a study with *Pouzolzia zeylanica* extracts, cold ethyl extract was found to be most powerful scavenger of hydroxyl radicals.[24] In the context of these literature reports, the observation made in the present study of *Adhatoda vasica* leaf extracts exhibiting Hydroxyl radical-scavenging activity gains and signifies in strengthening the antioxidant potential of the leaves.

Plants with iron chelating activity are most effective for lipid peroxidation reaction and therefore play a key role in medicinal practice. A study finding from *Toona sinensis* extracts and gallic acid and the results reported on celecoxib and amtolmetin guacyl possess antioxidant and metal-chelating abilities, which might contribute to their beneficial anti-inflammatory effects.[25,26] The ethyl acetate and isopropanol leaf extracts of *Adhatoda vasica* exhibited high levels of chelating property followed by hexane extract.

The rhizome extract of *Dioscorea alata* possessed radical scavenging activity and showed protective effect on calf thymus DNA and plasmid DNA as evaluated by Ethidium Bromide.[27] The alcohol: water (1:1) extract of curry leaves (Murraya koenigii L.) showed the highest antioxidant as reflected by the inhibition of ferrous sulfate: ascorbate-induced fragmentation and sugar oxidation of calf thymus DNA.[28]

5. CONCLUSION:

Body can handle free radicals normally by means of antioxidant system, but the overproduction of free radicals attacks the macromolecules leading to cellular damage and homeostatic disruption. Plants have been an important source of medicine and plants derived drugs have been helped humans in the maintenance of health for thousands of years. Free radical scavenging property of antioxidants provides protection to the living organisms from damage caused by uncontrolled production of free radicals and prevents homeostatic disruption.

Our results showed that the *Adhatoda vasica* leaves possessed considerable level of antioxidants radical scavenging activity. The results revealed that the DNA damage was effectively counteracted by the *Adhatoda vasica* leaf extracts. The phytochemical analysis of *Adhatoda vasica* has revealed the presence of compounds including flavonoids, phenols and alkaloids.

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